Antimicrobial Activity of Essential Oil and Various Extracts of Fruits of Greater Cardamom

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Agnihotri and Wakode: Antimicrobial Activity of *Amomum subulatum* Roxb

Greater cardamom (*Amomum subulatum* Roxb. Zingiberaceae) commonly known as "*Bari ilaichi*" is a well known plant used in Ayurvedic and Unani medicine. It has been used for the treatment of various diseases and disorders like gastric ulcer. Therefore antimicrobial activity of petroleum ether, methanol and aqueous extracts from leaves and roots, essential oil and isolated vasicine from *A. vasica* were tested against various microorganisms. Antimicrobial activity was done by disc diffusion method. The zone of inhibition observed was compared with that of standard drugs, ciprofloxacin and fluconazole. Minimum inhibitory concentration was determined against microorganisms used in the study. The results of this study reveal that methanol extract of fruits of *A. subulatum* shows remarkable antimicrobial activity against *Escherichia coli* whereas in case of other microorganisms used it was found inferior to the standard drug used. Methanol extract of rind showed good antimicrobial activity against *Staphylococcus aureus*. It was found that the essential oil isolated was effective against majority of microorganisms used viz. *Bacillus pumilus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*.

Key words: *Amomum subulatum*, essential oil, minimum inhibitory concentration, zone of inhibition

Amomum is a genus of terrestrial, rhizomatous herb, distributed chiefly in Africa and tropical Asia, found in the eastern Himalayas and cultivated in Nepal, northern West Bengal, Sikkim and Assam hills[1]. The seeds are reported to possess stimulant, stomachic,

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TABLE 1: ANTIMICROBIAL ACTIVITY OF *AMOMUM SUBULATUM* ROXB.

Microorganism	Zone of Inhibition (mm)			
	STD	ASR	ASF	ASO
Gram positive bacteria				
Bacillus pumilus	17	16.7	17	20
Bacillus subtilis	18	15	16	17
Staphylococcus aureus	15	15	20	15
Micrococcus luteus	17	12	15	14
Staphylococcus epidermidis	20	17	16	20
Gram negative bacteria				
Escherichia coli	20	20.3	18	18.5
Pseudomonas aerugenosa	16	15	16	16
Fungal strains				
Candida albicans	19	14	15	15
Aspergillus niger	20	19	15	17
Sachharomyces cerevisiae	16	15	15	16

ASR is *A. subulatum* rind, ASF is *A. subulatum* fruits, ASO is essential oil of *A. subulatum*, standard drug used for bacteria is ciprofloxacine and for fungi is fluconazole, (---) indicates no activity.

alexipharmic and astringent properties, and are used in folklore medicine for the treatment of indigestion, vomiting, biliousness, abdominal pains and rectal diseases. The seeds are found to promote elimination of bile and are used to treat congestive jaundice; they are also used in gonorrhea, while the pericarp has been reported to be useful in treating headache and stomatitis. The aromatic oil extracted from the seeds is applied to the eyes in cases of inflammation[1].

The seeds contain the glycosides, petunidin-3,5-diglucoside and leucocynidin-3-O-β-D-glucopyranoside, and a new aurone glycoside, subulin. Acid hydrolysis of subulin gives the aglycone, subulaurone. The presence of chalcone, cardamonin and a flavanone, alpinetin was also reported[1]. The seeds on steam distillation yield a dark brown, mobile essential oil (2.5%) having a characteristic odor of cineol[1]. Essential oil of *A. subulatum* has been studied extensively for its composition and its antimicrobial potential[2- 7].

In the present study, separate extracts of seeds and rind of fruit of *A. subulatum* and essential oil isolated from the whole fruit were screened for antibacterial and antifungal potential.

Amomum subulatum (dried fruits) was procured from the local market of Sunder Nagar, Distt. Mandi, Himachal Pradesh, India. The crude drug was identified and authenticated at the National Bureau of Plant and Genomic Research (NBPGR), New Delhi.

The fruits and rind of *Amomum subulatum* Roxb. were separated and subjected to extraction separately. Using a Soxhlet apparatus, the powdered parts of crude drug were extracted using 80% methanol as the solvent. The obtained extracts were concentrated under reduced pressure. To isolate the essential oil, the plant material was hydrodistilled in an all-glass Clevenger's apparatus. The isolated oil was kept in air tight glass bottles in a refrigerator.

The antimicrobial activity was evaluated using the disc diffusion technique[8]. Filter paper discs of 6 mm diameter were prepared using Whatman No.1 filter paper. The discs were sterilized by autoclaving for 20 min at 15 lbs pressure. Then the discs were soaked in methanol extracts to obtain a concentration of 1 mg/disc. The Petri dishes, antibiotic assay medium 1 (for bacteria) and potato dextrose agar medium (for fungi) were sterilized by autoclaving. *Bacillus pumilus, Bacillus subtilis, Stephylococcus aureus, Micrococcus epidermidis, Stephylococcus epidermidis, Escherichia coli, Pseudomonas aerugenosa*, and fungal strains *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* were used for the study. One milliliter of one day old bacterial culture was added to the sterilized medium. The cultures were inoculated, stirred well and this medium was added to the Petri dishes. The filter paper discs impregnated with plant extracts and essential oil were placed aseptically and carefully on the solidified medium. The plates were kept in a refrigerator for proper diffusion of extracts. Then the plates were incubated at 37º for 24 h and 28º for 72 h for bacteria and fungi, respectively. Afterwards the plates were observed for clear zone of inhibition. The results obtained were compared with those obtained with standards ciprofloxacin and fluconazole for bacteria and fungi, respectively.

Minimum inhibitory concentration (MIC) was estmated for the microorganisms selected for the study[9]. MIC was determined by disc diffusion assay. Sterile filter paper discs (6 mm diameter) containing 2.5 to 1000 µg/disc of plant extracts were placed on the surface of a medium. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar.

In the present study, 80% methanol extracts of *A. subulatum* (rind 12.93%, fruit 15.94%) and

essential oil from whole fruits (0.8%) were tested for antimicrobial potential. Table 1 shows the zone of inhibition in mm of methanol extracts of rind and fruits and essential oil of *A. subulatum* against microorganisms used.

Methanol extract of rind of *A. subulatum* showed remarkable antimicrobial activity against *E. coli*. When compared with ciprofloxacin, it was found equivalent in potency against *S. aureus*, whereas slight inferior against remaining bacterial stains. Methanol extract of fruits of *A. subulatum* was found to be effective against *S. aureus*. It was found equivalent to standard drug against *B. pumilus* and *P. aerugenosa*.

Essential oil isolated from whole fruits showed good results against *B. pumilus*. It was found equivalent to the standard used against *S. epidermidis*, *P. aerugenosa* and *S. cerevisiae*. It was found that methanol extracts of fruit and rind as well as essential oil all possesses antifungal activity but less than standard drug used.

It can be seen from the results obtained for MIC that rind extract of *A. subulatum* is having lower values as compared to fruit extract of *A. subulatum* in majority of microorganisms. Methanol extract of rind was showing exceptionally lower MIC in case of *S. aureus* (10 µg/disc).

The results obtained in the present study revealed that methanolic extracts of fruit and rind of *A. subulatum* possessed broad spectrum antimicrobial activity. The essential oil isolated from the whole fruits shows good antimicrobial activity against microorganisms used in the study.

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